

Cytokines, chemokines, and AnxA1 quantification

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An abbreviated version of this protocol was published in eLIFE in Mar 2022
Targeting the Annexin A1-FPR2/ALX pathway for host-directed therapy in dengue disease
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Detailed protocol

Dear Wei Li,

Thank you for your question.

Tissue homogenates were obtained following a previously described protocol (Amaral et al., 2011; Coelho et al., 2008; Vieira et al., 2017).

Briefly, tissues were harvested at indicated time points and stored at -20 °C until analysis. Upon thawing, tissues (100mg of tissue/ml of extraction buffer) were homogenized in PBS containing protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride – PMSF, 0.1 mM benzethonium chloride, 10 mM EDTA, and 20 K I aprotinin A), 0.05% Tween 20 and 0.5% bovine serum albumin (BSA). Samples were homogenised in bead mill homogeniser (TissueLyser LT; Qiagen). Samples were then centrifuged for 10 min at 10,000 rpm, and the supernatant was stored at -20 °C until use in enzyme-linked immunoassays (ELISA). Samples were diluted at 1:2 or 1:3 in BSA/PBS, and cytokine levels were assessed by ELISA according to the manufacturer instructions.

I hope this helps you in your research.

All the best,

Dr Michelle Sugimoto

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